REMARKS/ARGUMENTS

Claims 1-5, 7-14, 19-34, 36-43, and 48-56 are pending.

Claims 1, 9, 28-30, and 38 have been amended.

Claims 6, 15-18, 35, and 44-47 have been cancelled.

Support for the amendments is found in the claims and specification (e.g., the Examples at pages 39-40, bridging paragraph) as originally filed. No new matter is believed to have been added.

Claims 1-2, 4-5, 7, 9, 11-14, 19, 21, 24-25, 27-31, 33-38, 40-50, and 56 are rejected under 35 U.S.C. 103(a) over Misako et al., JP 2003-174900, and Schleper et al., *J. Bicteriol.*, 179(24):7803-7811 (1997). The rejection is traversed because the combination of the references does not teach that the temperature of a regeneration region is controlled at 30-40 °C.

Misako describes a circulating amplification process and an amplifier having a flow channel and a heat-at-high-temperate chamber, a low temperature chamber, and a moderate temperature chamber (fig. 3). Misako describes using "heating at high temperature section 5" (fig. 3), i.e., a usual PCR process utilizing a thermostable polymerase (e.g., amplifying at around 72 °C) because the chamber is set at that temperature.

Misako does not teach a regeneration region chamber controlled at 30 to 40 °C, which is too low for the DNA polymerase of Misako. Using a low optimal temperature synthetase is advantageous because enzymes that are not used in a conventional PCR (e.g., described by Misako) can be used to improve reliability of the amplification (see the paragraph bridging pages 8-9).

Further, a polymerase having a lower optimum temperature, e.g., a DNA polymerase Klenow fragment from *E.coli* having an optimum temperature of around 37 °C, cannot be used in the Misako PCR. Specifically, in a conventional solution PCR, the time-lag might

occur for the enzyme having a lower optimum temperature to go into activation from the denaturing conditions. However, in combination with the flow-type PCR, a DNA polymerase having a lower optimum temperature (e.g., 30-40 °C) is ready to do its task because it is sealed in an optimum condition at the beginning of the regeneration region. Because of the lower temperature condition in which the enzyme is immobilized, the enzyme is more stable than that in the Misako PCR. Stability of the enzyme is more likely to be achieved at a lower temperature, if compared to an enzyme having an optimum temperature at around 72 °C of the Misako PCR.

Moreover, the Schleper polymerase is not described to be used in PCR. There is no disclosure in Schleper that an amplification of DNA can be accomplished reliably with the *Cenarchaeum symbiosum* polymerase. Schleper only discloses that *C. symbiosum* DNA polymerase exhibits its highest specific activity with a gapped-duplex DNA as a substrate (abstract, fig. 4), but does not teach using the isolated enzyme for DNA amplification such as disclosed in Misako. Also the optimum temperature of the polymerization activity on the gapped-duplex DNA of the Schleper enzyme is 42°C, while its 3'-5'-*exonuclease activity* temperature is 38 °C (fig. 4, and page 7807, right col) (see claims 1-5, 7-14, and 19-27).

There is no motivation of using the Schleper enzyme at 30-40 °C in the third chamber of the Misako amplification device. Indeed, one would not have used Schleper's enzyme in a regeneration chamber controlled at 30-40 °C because of the enzyme's high exonuclease activity 38 °C. Thus, Misako and Schleper do not make the claimed invention obvious. Applicants request that the rejection be withdrawn.

Claims 3, 10, 13, 20, 22-23, 32, 39, and 51-54 are rejected under 35 U.S.C. 103(a) over Misako et al., JP 2003-174900, Schleper et al., *J. Bicteriol.*, 179(24):7803-7811 (1997), and Moses, Mol. And Cellular Biol., 14(4):2767-2776 (1994).

Claims 5 and 12-14 are rejected under 35 U.S.C. 103(a) over Misako et al., JP 2003-174900, Schleper et al., *J. Bicteriol.*, 179(24):7803-7811 (1997), and Hideo, JP 6-30776.

Claim 8 is rejected under 35 U.S.C. 103(a) over Misako et al., JP 2003-174900, Schleper et al., *J. Bicteriol.*, 179(24):7803-7811 (1997), and Southgate, US 5,863,801.

Claims 26 and 55 are rejected under 35 U.S.C. 103(a) over Misako et al., JP 2003-174900, Schleper et al., *J. Bicteriol.*, 179(24):7803-7811 (1997), and Belford, *Biotech. Bioengineering*, 33:1047-66 (1989).

The rejection is traversed because combinations of the references do not teach that the temperature of a regeneration region is controlled at 30-40 °C.

The disclosures of Misako and Schleper are discussed above. Moses, Hideo, Southgate, and Belfort do not cure the deficiency of Misako and Schleper.

Moses teaches that a DNA polymerase bound to an affinity column is active (e.g., fig. 2 and page 2768, right col.).

Hideo teaches carrying an amplification reaction by transporting a reaction solution as a mobile phase through various reaction sections (fig. 1-2, abstract).

Southgate describes a device and a method for automatic isolation of a nucleic acid comprising pumps that can reverse the flow of reagents (col. 20, lines 11-25).

Belfort describes membrane bioreactors (e.g., page 1051-53).

However, Moses, Hideo, Southgate, and Belfort do not teach or suggest using a low temperature synthetase for amplification and an amplifier having a regeneration region controlled at 30-40 °C.

Thus, the cited references do not make the claimed invention obvious. Applicants request that the rejection be withdrawn.

A Notice of Allowance for all pending claims is requested.

Respectfully submitted,

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